AMENDMENTS

In the Specification:

Please amend the paragraph at page 2, lines 22-28, as follows:

B1

Many such automated systems are commercially available. For example, a variety of automated systems are available from the Zymark Corporation (Zymark Center, Hopkinton, MA), which utilize various Zymate systems (*see also*, ~~http://www.zymark.com/~~ the world wide web at zymark.com), which typically include, e.g., robotics and fluid handling modules. Similarly, the common ORCA® robot, which is used in a variety of laboratory systems, e.g., for microtiter tray manipulation, is also commercially available, e.g., from Beckman Coulter, Inc. (Fullerton, CA).

Please amend the paragraph at page 65, lines 7-29, as follows:

B2

BLAST is described in Altschul *et al., J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (~~http://www.ncbi.nlm.nih.gov/~~ the world wide web at ncbi.nlm.nih.gov). This algorithm first identifies high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al., supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100,

B2 cont'd.

M=5, N=-4, and a comparison of both strands. For amino acid (protein) sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

Please amend the paragraph at page 73, line 22, to page 74, line 13, as follows:

B3

The second basic method for acquiring nucleic acids does not rely on the physical pre-existence of a nucleic acid. Instead, nucleic acids are generated synthetically, e.g., using well-established nucleic acid synthesis methods. For example, nucleic acids can be synthesized using commercially available nucleic acid synthesis machines which utilize standard solid-phase methods. Typically, fragments of up to about 100 bases are individually synthesized, then joined (e.g., by enzymatic or chemical ligation methods, or polymerase mediated recombination methods) to form essentially any desired continuous sequence or sequence population. For example, the polynucleotides and oligonucleotides of the invention can be prepared by chemical synthesis using, *e.g.*, the classical phosphoramidite method described by Beaucage *et al.*, (1981) Tetrahedron Letters 22:1859-69, or the method described by Matthes *et al.*, (1984) EMBO J. 3: 801-05., e.g., as is typically practiced in automated synthetic methods. According to the phosphoramidite method, oligonucleotides are synthesized, *e.g.*, in an automatic DNA synthesizer, assembled and, optionally, cloned in appropriate vectors. In addition, essentially any nucleic acid can be custom ordered from any of a variety of commercial sources, such as The Midland Certified Reagent Company (mcrc@oligos.com), The Great American Gene Company (~~http://www.genco.com~~ the world wide web at genco.com), ExpressGen Inc. (~~www.expressgen.com~~ the world wide web at expressgen.com), Operon Technologies Inc. (Alameda, CA) and many others. Similarly, peptides and antibodies (useful in various embodiments noted below) can be custom ordered from any of a variety of sources, such as PeptidoGenic (pkim@ccnet.com), HTI Bio-products, inc. (~~http://www.htibio.com~~ the world wide web at htibio.com), BMA Biomedicals Ltd (U.K.), Bio.Synthesis, Inc., Research Genetics (Huntsville, Alabama) and many others.

Please amend the paragraph at page 93, lines 11-20, as follows:

B4

Amplifiers typically include some sort of heating element and can also include a cooling element. Such elements commonly include (but are not limited to) resistive elements, programmable resistors, micromachined zone heating chemical amplifiers, Peltier solid state heat pumps (*see, e.g.,* ~~http://pw1.netcom.com/~sjnoll/peltier.html~~ //pw1.netcom.com/~sjnoll/peltier.html (via hypertext transport protocol), heat pumps, resistive heaters, refrigeration units, heat sinks, Joule Thompson cooling devices, a heat exchanger, a hot air blower, etc. Any of the above elements are optionally operably coupled to a computer comprising a set of instructions which directs or instructs the elements in the amplification process, e.g., according to user input data or computer calculated predictions.

Please amend the paragraph at page 93, line 21 to page 94, line 2, as follows:

B5

Recently, attempts have been made to shorten the time required for each cycle of PCR, an advantage in the present method, in that reduction in this time increases the overall throughput of the system. Such methods often reduce the time by, for example, performing the PCR in devices that allow rapid temperature changes. The use of apparatus that allow greater heat transfer, *e.g.*, incorporating thin-walled tubes, turbulent air-based machines, and the like also facilitate the use of shorter cycle times. For example, the RapidCycler™, from Idaho Technologies, Inc. (~~http://www.idahotech.com/~~ the world wide web at idahotech.com Salt Lake City, UT) allows relatively rapid ramping times between each temperature of a PCR and relatively efficient thermal transfer from the cycler to the samples. Similarly, the RAPID (Ruggedized Advanced Pathogen Identification Device) from Idaho Technologies, Inc. provides a thermal cycler with concurrent fluorescence monitoring to speed analysis as well.

Please amend the paragraph at page 100, line 29 to page 101, line 31, as follows:

B6

An alternative to TaqMan is the use of molecular beacons to assess library quality. Molecular beacons are oligonucleotide probes that can report the presence of specific nucleic acids in homogeneous solutions (Tyagi and Kramer (1996) "Molecular beacons: probes that fluoresce upon hybridization." Nat Biotechnol 14, 303-308. They are used for real-time monitoring of PCR or other amplification reactions and for the detection of RNAs within living cells. Molecular beacons are hairpin-shaped molecules with an internally quenched fluorophore whose fluorescence

is restored when they bind to a target nucleic acid (*see* Tyagi and Kramer, *id*). They are designed so that the loop portion of the molecule is a probe sequence complementary to a target nucleic acid molecule. The stem is formed by an annealing of complementary arm sequences on the ends of the probe sequence. A fluorescent moiety is attached to the end of one arm and a quenching moiety is attached to the end of the other arm. The stem keeps these two moieties in close proximity to each other, causing the fluorescence of the fluorophore to be quenched by energy transfer. When the probe encounters a target molecule, it forms a hybrid that is longer and more stable than the stem hybrid and its rigidity and length preclude the simultaneous existence of the stem hybrid. Thus, the molecular beacon undergoes a spontaneous conformational reorganization that forces the stem apart, and causes the fluorophore and the quencher to move away from each other, leading to the restoration of fluorescence which can be detected. Further details on Molecular Beacons and their use can be found at ~~http://www.molecular-beacons.org~~ the world wide web at molecular-beacons.org and in the following references: Tyagi et al. (1998) "Multicolor molecular beacons for allele discrimination" Nat Biotechnol 16:49-53; Matuso (1998) "In situ visualization of mRNA for basic fibroblast growth factor in living cells" Biochimica Biophysica Acta 1379:178-184; Sokol et al. (1998) "Real time detection of DNA-RNA hybridization in living cells" Proc Natl Acad Sci USA 95:11538-11543; Leone et al. (1998) "Molecular beacon probes combined with amplification by NASBA enable homogeneous, real-time detection of RNA" Nucleic Acids Res ~~26, 2150-2155~~ 26:2150-2155; Piatek et al. (1998) "Molecular beacon sequence analysis for detecting drug resistance in Mycobacterium tuberculosis" Nat Biotechnol 16:359-363; Kostrikis et al. (1998) "Spectral genotyping of human alleles" Science 279:1228-1229; Giesendorf et al. (1998) "Molecular beacon: a new approach for semiautomated mutation analysis" Clin Chem 44:482-486; Marras et al. (1999) "Multiplex detection of single-nucleotide variations using molecular beacons" Genet Anal 14:151-156; and Vet at al. (1999) "Multiplex detection of four pathogenic retroviruses using molecular beacons" Proc Natl Acad Sci USA 96:6394-6399.

B6 cont.

Please amend the paragraph at page 106, lines ~~16~~ 14-22, as follows:

B7

The charged complexes formed by the protocols outlined above are readily sorted by electrophoretic mobility to remove uncomplexed material. Dispensing these particles into separate wells of a microtiter plate uses, e.g., electrophoresis, e.g., in which the particles travel down a

B7 Con't

capillary (or channel) in single file, much like in a FACS machine (or chip). A fluorescent detector (e.g., LIF, confocal laser with suitable PMT/CCD) set up at the end of the system detects passage of particles and directs particles into the receiving well. Flow cytometry systems which will sort into microtiter plates of any format, are available, e.g., from Cytomation (~~http://www.cytomation.com/~~ the world wide web at cytomation.com; Fort Collins, CO).

Please amend the paragraph at page 114, lines 7-15, as follows:

B8

Various systems are also available for simultaneous synthesis and folding of complex proteins. For example, the control of redox potential, the use of helper proteins (from both bacterial and eukaryotic systems) and the like can be used to provide for improved cell free translation. Optionally, proteins may be added which aid in protein refolding, such as by maintaining solubility of the nascent or partially folded protein (e.g., chaperonins) or by adjusting the configuration of inter- and intra- molecular disulfide bonds (e.g. protein disulfide isomerase). In addition to the references noted above, additional details regarding cell free protein translation can be found via hyptertext transport protocol at ~~http://chemeng.stanford.edu/html/swartz.htm~~ chemeng.stanford.edu/html/swartz.html.

Please amend the paragraph at page 126, line 25 to page 127, line 20, as follows:

B9

The product identification module can include detection and/or selection modules which facilitate detection or selection of array members. Such modules can include, e.g., an array reader which detects one or more member of the array of reaction products. Array readers are commercially available, generally constituting a microscope or CCD and a computer with appropriate software for identifying or recording information. In particular, array readers which are designed to interface with standard microtiter trays and other common array systems are commercially available. In addition to product manufacturer information from many of the various product manufacturers noted herein, detection protocols and systems are well known. For example, basic bioluminescence methods and detection methods which describe e.g., detection methods include LaRossa Ed. (1998) Biolumniescence Methods and Protocols: Methods in Molecular Biology Vol. 102, Humana Press, Towata, NJ. Basic Light microscopy methods, including digital image processing is described, e.g., in Shotton (ed) (1993) Electronic Light Microscopy:

B9
cont

Techniques in Modern Biomedical Microscopy Wiley-Liss, Inc. New York, NY. Fluorescence Microscopy methods are described, e.g., in Hergman (1998) Fluorescence Microscopy Bios Scientific Publishers, Oxford, England. Specialized imaging instruments and methods for screening large numbers of images have also been described, e.g., "MICROCOLONY IMAGER INSTRUMENT FOR SCREENING CELLS EXPRESSING MUTAGENIZED ENZYMES" U.S. Pat. No. 5,914,245 to Bylina et al.; "ABSORBTION SPECTRA DETERMINATION METHOD FOR HIGH RESOLUTION IMAGING MICROSCOPE…" U.S. Pat. No. 5,859,700 to Yang; "CALIBRATION OF FLUORESCENCE RESONANCE ENERGY IN MICROSCOPY…" WO 9855026 (Bylina et al.); "OPTICAL INSTRUMENT HAVING A VARIABLE OPTICAL FILTER" Yang and Youvan U.S. Pat. No. 5,852,498; Youvan (1999) "Imaging Spectroscopy and Solid Phase Screening" IBC World Congress on Enzyme Technologies and the world wide web at kairos.com ~~http://www.kairos.com~~;. These systems can be incorporated into the present invention to provide high-throughput screening systems.

Please amend the paragraph at page 137, lines 10-18, as follows:

B10

The reactant arrays of the invention can be either physical or logical in nature. For the generation of common arrangements involving fluid transfer to or from microtiter plates, a fluid handling station is used. Several "off the shelf" fluid handling stations for performing such transfers are commercially available, including e.g., the Zymate systems from Zymark Corporation (Zymark Center, Hopkinton, MA; the world wide web at zymark.com ~~http://www.zymark.com/~~) and other stations which utilize automatic pipettors, e.g., in conjunction with the robotics for plate movement (e.g., the ORCA® robot, which is used in a variety of laboratory systems available, e.g., from Beckman Coulter, Inc. (Fullerton, CA).

Please amend the paragraph at page 137, lines 19-29, as follows:

B11

In an alternate embodiment, fluid handling is performed in microchips, e.g., involving transfer of materials from microwell plates or other wells through microchannels on the chips to destination sites (microchannel regions, wells, chambers or the like). Commercially available microfluidic systems include those from Hewlett-Packard/Agilent Technologies (e.g., the HP2100 bioanalyzer) and the Caliper High Throughput Screening System (*see, e.g.*,

B11 cont'd

~~http//www.calipertech.com/products/index.htm~~ the world wide web at calipertech.com/products/index.html). The Caliper High Throughput Screening System provides an interface between standard library formats and chip technologies (*see, e.g.,* the world wide web at calipertech.com ~~http://www.calipertech.com~~). Furthermore, the patent and technical literature includes examples of microfluidic systems which can interface directly with microwell plates for fluid handling.

Please amend the paragraph at page 137, line 30 to page 138, line 5, as follows:

B12

Thus, generally, microfluidic systems are commercially available. In addition, university groups such as Mark Burns' research group at The University of Michigan also describe various microfluidic systems (~~http://dow3029-mac5.engin.umich.edu/~~ dow3029-mac5.engin.umich.edu/ (via hypertext transport protocol); ~~http://www.engin,umich.edu/dept/cheme/people/burns.html~~ the world wide web at engin.umich.edu/dept/cheme/people/burns.html;~~ http://dow3029-mac5.engin.umich.edu/~~ dow3029-mac5.engin.umich.edu/ (via hypertext transport protocol). Accordingly, general fabrication principles and the use of various microfluidic systems is known and can be applied to the integrated systems of the present invention.

Please amend the paragraph at page 166, line 28 to page 167, line 13, as follows:

B13

Recently, methods for extracting nucleic acids at high yield from microbial cultures, broths, pathogen and environmental samples have been described. Where complex, soil-containing or mixed culture systems are targeted for characteriation or gene mining, these methods generally use any of a variety of treatments to provide high yield, high purity nucleic acids. For example, a variety of publications and patents describing such methods are listed herein. Examples include Short "PRODUCTION OF ENZYMES HAVING DESIRED ACTIVITIES BY MUTAGENESIS" U.S. Pat. 5,939,250 (*See also* the world wide web at accessexcellence.com/AB/IWT/1297xtremo.html ~~http://www.accessexcellence.com/AB/IWT/1297xtremo.html~~ and at diversa.com/techplat/techover.asp ~~http://www.diversa.com/techplat/techover.asp~~), Thompson, et al. (1998) "METHODS FOR GENERATING AND SCREENING NOVEL METABOLIC

B13
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PATHWAYS" United States Patent 5,824,485 and 6,783,431; and Carlson, et al. (1999) "METHOD OF RECOVERING A BIOLOGICAL MOLECULE FROM A RECOMBINANT MICROORGANISM" United States Patents 5,908,765, 5,837,470 and 5,773,221, which allege various methods for creating libraries from, e.g., uncharacterized heterogeneous microbial samples. The present invention provides, e.g., for automation, spatial or logical arrays and associated tools in mediating, improving or replacing these processes.